Standard Operating Procedure

Title:	MP10_02 – Blood Culture Collection Procedure

1. PURPOSE

The purpose of this procedure is to provide instruction on how to collect a blood culture. There are instructions below for collecting a blood culture from a venipuncture, from a fresh IV start, and from an indwelling central venous access device.

2. SCOPE

2.1. This procedure applies to all staff members who are responsible for collecting blood cultures.

3. PRINCIPLE

3.1. The blood of healthy individuals is normally sterile; therefore the presence of microorganisms in the blood is of utmost diagnostic importance. When microorganisms multiply in the blood stream at a rate faster than the reticuloendothelial system can remove them, the result is a state of bacteremia. The presence of microorganisms in a patient's blood is life threatening and is associated with a significant mortality rate. Rapid detection, identification and susceptibility testing of blood culture isolates is one of the most important functions of the Clinical Microbiology Laboratory.

3.2. The BacT/ALERT® 3D Microbial Detection System is used to detect the presence of microorganisms in blood of patients suspected of having bacteremia. The liquid media in the collection bottles provides optimal nutritional requirements for organisms commonly found in blood infections, while the instrument provides the environmental conditions needed for growth. Bottles are read every ten minutes and processed through the software algorithms to measure signs of growth.

4. REAGENTS, MATERIALS & EQUIPMENT

Venipuncture	Fresh IV Start	Central Line / Port
Gloves and other PPE as required	Gloves and other PPE as required	Gloves and other PPE as required
Tourniquet	IV Start Kit	Alcohol Prep Pads
Chloraprep Skin Disinfectant	Alcohol Prep Pads	Needleless Adapter (cap)
Sterile Gauze	Sharpie Permanent Marker	NS Flush Syringes
Alcohol Prep Pads	Blood Culture Bottles	10mL Syringe
Sharpie Permanent Marker	SteriPath Luer Collection Device	Sharpie Permanent Marker
Blood Culture Bottles	Biohazard Bag	Blood Culture Bottles
SteriPath Collection Device		SteriPath Luer Collection Device
Tape/Band Aid		Biohazard Bag
Biohazard Bag		

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5. PROCEDURAL NOTES:

5.1. Skin Preparation/Decontamination is critical. Do not deviate from stated protocol.

5.2. Blood Volume is extremely important. Follow instructions regarding optimal blood volume.

5.3. Bottles must be labeled with the barcode oriented **vertically**.

5.4. Bottles must be labeled with the specific location of the draw (Hand, Arm, AC, FA, PICC, etc).

5.5. The SteriPath device should not be used on pediatric patients less than age 18.

6. QUALITY CONTROL

6.1. Bottles are stored in the microbiology laboratory. Inventory is rotated to ensure that bottles are used in order of expiration date.

6.2. Certificates of Conformance are maintained from each lot number of media in use certifying that samples were tested by standard procedures including the methods and control cultures specified in the CLSI approved standard. No additional QC is required by the end user.

6.3. Visual check of the integrity of bottles is also monitored upon receipt and prior to use.

7. BLOOD CULTURE COLLECTION - VENIPUNCTURE

7.1. NOTE: See special note below regarding collecting blood from a patient who is on Isolation Precautions.

7.2. Ensure that all supplies required to collect a blood culture are laid out, ready for use and within easy reach.

7.3. Inspect broth and sensor on bottom of bottles. Insure that broth is clear, and sensor on the bottom of bottle is intact and has a green color. Do not use bottles in which the broth is cloudy, if the sensor is off-color (i.e., yellow), if there are any visible cracks in the bottle or if expiration date has past.

7.4. Using a sharpie marker, mark the maximum fill volume on each bottle to be collected. For the Standard Aerobic and Anaerobic bottles, the maximum fill volume is 10 mL of blood – place a mark 8-10 mL above the liquid line. For Pediatric bottles, the maximum fill volume is 4 ml – place a mark 3-4 mL above the liquid line.

Note: Some newer bottles have a Fill To Line indicated on the bottle label. Look for the WHITE LINE etched onto the label near the picture of the bottle with the word "fill". The fill to line is the white line, NOT the small black arrow inside the bottle picture.

7.5. Remove the plastic top on the bottles and cleanse the hub with an alcohol prep pad for 10-15 seconds. Leave the alcohol pad on top of the bottle until ready to use.

7.6. Using clean disposable gloves, apply tourniquet and locate a suitable vein. Once a suitable vein has been located, remove the tourniquet.

7.7. Thoroughly cleanse the skin with ChloraPrep – **DO NOT DEVIATE from protocol**:

7.7.1. Remove the ChloraPrep applicator and squeeze the handle together to break the ampule. Do not touch the sponge.

7.7.2. Prime the sponge by pressing it against the patient's skin several times to ensure that the sponge is wet with the disinfectant.

7.7.3. Clean the site with ChloraPrep applicator for **30 seconds** – scrubbing back and forth across the arm in two different directions.

7.7.4. Allow the disinfectant to **dry for 30 seconds** before drawing the blood culture. This will allow maximum effectiveness of the disinfectant.

7.7.5. Do not touch skin after cleaning has been performed.

7.8. Select the appropriate SteriPath needle size and open the SteriPath device packaging.

7.9. Check the connection between the needle tubing and the device to make sure it is tight.

7.10. Reapply tourniquet and perform venipuncture procedure. Observe the flash of blood into the needle tubing before continuing.

7.11. Gently press the plunger forward to fill the waste chamber.

7.12. Once the waste chamber is full, press the plunger all the way forward until you hear a click and you cannot press forward any more.

7.13. Insert the aerobic bottle up inside the adapter cap and press the bottle up onto the needle until the blood begins to flow. Carefully watch blood flowing into the bottle and remove the bottle when the volume approaches your "fill to" mark. Invert the bottle 3-4 times to mix. Repeat the process with the anaerobic bottle.

7.14. Once blood collection is complete, remove the tourniquet and place a piece of gauze over the venipuncture site. Retract the needle out of the patient's arm, and press the gauze firmly over the site and hold to stop the bleeding. Discard the entire SteriPath device into a sharps container.

7.15. When venipuncture is complete, properly label each bottle identifying the patient and the collection date/time/site. Apply labels to the bottles vertically.

7.16. Place bottles into a biohazard bag and transport to the laboratory.

7.17. NOTE: Avoid drawing blood through indwelling intravenous or intra-arterial catheters unless blood cannot be obtained by venipuncture, or unless the diagnosis of catheter sepsis is suspected.

7.18. Finger sticks and cultures drawn from the foot are unacceptable for blood cultures.

8. BLOOD CULTURE COLLECTION – FRESH IV START

8.1. NOTE: See special note below regarding collecting blood from a patient who is on Isolation Precautions.

8.2. Ensure that all supplies required to collect a blood culture are laid out, ready for use and within easy reach.

8.3. Inspect broth and sensor on bottom of bottles. Insure that broth is clear, and sensor on the bottom of bottle is intact and has a green color. Do not use bottles in which the broth is cloudy, if the sensor is off-color (i.e., yellow), if there are any visible cracks in the bottle or if expiration date has past.

8.4. Using a sharpie marker, mark the maximum fill volume on each bottle to be collected. For the Standard Aerobic and Anaerobic bottles, the maximum fill volume is 10 mL of blood – place a mark 8-10 mL above the liquid line. For Pediatric bottles, the maximum fill volume is 4 ml – place a mark 3-4 mL above the liquid line.

Note: Some newer bottles have a Fill To Line indicated on the bottle label. Look for the WHITE LINE etched onto the label near the picture of the bottle with the word "fill". The fill to line is the white line, NOT the small black arrow inside the bottle picture.

8.5. Remove the plastic top on the bottles and cleanse the hub with an alcohol prep pad for 10-15 seconds. Leave the alcohol pad on top of the bottle until ready to use.

8.6. Using clean disposable gloves, apply tourniquet and locate a suitable vein. Once a suitable vein has been located, remove the tourniquet.

8.7. Open the IV Start Kit and lay out all supplies for easy reach.

8.8. Thoroughly cleanse the skin with ChloraPrep – **DO NOT DEVIATE from protocol**:

8.8.1. Remove the ChloraPrep applicator and squeeze the handle together to break the ampule. Do not touch the sponge.

8.8.2. Prime the sponge by pressing it against the patient's skin several times to ensure that the sponge is wet with the disinfectant.

8.8.3. Clean the site with ChloraPrep applicator for **60 seconds** – scrubbing back and forth across the arm in two different directions.

8.8.4. Allow the disinfectant to **dry for 30 seconds** before drawing the blood culture. This will allow maximum effectiveness of the disinfectant. Do not touch skin after cleaning has been performed.

8.9. Open the SteriPath Device with a Luer Connection packaging and check the connection between the needle tubing and the device to make sure it is tight.

8.10. Reapply tourniquet, insert the IV and hook up the SteriPath Luer tubing.

8.11. Gently press the plunger forward to fill the waste chamber.

8.12. Once the waste chamber is full, press the plunger all the way forward until you hear a click and you cannot press forward any more.

8.13. Insert the aerobic bottle up inside the adapter cap and press the bottle up onto the needle until the blood begins to flow. Carefully watch blood flowing into the bottle and remove the bottle when the volume approaches your "fill to" mark. Invert the bottle 3-4 times to mix. Repeat the process with the anaerobic bottle.

8.14. If other laboratory tests need to be collected, reinsert the small tube adapter inside the adapter cap and proceed to collect other vacuatiner tubes as needed.

8.15. Once blood collection is complete, discard the entire SteriPath device into a sharps container.

8.16. Finish securing and taping the IV line.

8.17. When venipuncture is complete, properly label each bottle identifying the patient and the collection date/time/site. Apply labels to the bottles vertically.

8.18. Place bottles into a biohazard bag and transport to the laboratory.

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9. BLOOD CULTURE COLLECTION – COLLECTION FROM CENTRAL LINE

9.1. NOTE: See special note below regarding collecting blood from a patient who is on Isolation Precautions.

9.2. NOTE: Refer to nursing protocol regarding the collection of blood cultures from a Central Line.

9.3. Refer to the policy on Intravascular Therapy and Devices in the Patient Care Manual for additional information regarding the maintenance of Intravascular Devices.

9.4. Ensure that all supplies required to collect a blood culture are laid out, ready for use and within easy reach.

9.5. Inspect broth and sensor on bottom of bottles. Insure that broth is clear, and sensor on the bottom of bottle is intact and has a green color. Do not use bottles in which the broth is cloudy, if the sensor is off-color (i.e., yellow), if there are any visible cracks in the bottle or if expiration date has past.

9.6. Using a sharpie marker, mark the maximum fill volume on each bottle to be collected. For the Standard Aerobic and Anaerobic bottles, the maximum fill volume is 10 mL of blood – place a mark 8-10 mL above the liquid line. For Pediatric bottles, the maximum fill volume is 4 ml – place a mark 3-4 mL above the liquid line.

Note: Some newer bottles have a Fill To Line indicated on the bottle label. Look for the WHITE LINE etched onto the label near the picture of the bottle with the word "fill". The fill to line is the white line, NOT the small black arrow inside the bottle picture.

9.7. Remove the plastic top on the bottles and cleanse the hub with an alcohol prep pad for 10-15 seconds. Leave the alcohol pad on top of the bottle until ready to use.

9.8. Stop all infusions.

9.9. Scrub the cap vigorously for 15 seconds with an alcohol pad and allow to air dry.

9.10. Flush with 10mL Normal Saline.

9.11. REMOVE needleless adapter cap prior to collecting a blood culture.

9.12. Scrub the cap again for 15 seconds with an alcohol pad and allow to air dry.

9.13. Attach the SteriPath Blood Culture Collection Device with Luer Connection to the hub. Ensure that all tubing connections on the device are tight and secure.

9.14. Gently press the plunger forward to fill the waste chamber.

9.15. Once the waste chamber is full, press the plunger all the way forward until you hear a click and you cannot press forward any more.

9.16. Insert the aerobic bottle up inside the adapter cap and press the bottle up onto the needle until the blood begins to flow. Carefully watch blood flowing into the bottle and remove the bottle when the volume approaches your "fill to" mark. Invert the bottle 3-4 times to mix. Repeat the process with the anaerobic bottle.

9.17. If other laboratory tests need to be collected, reinsert the small tube adapter inside the adapter cap and proceed to collect other vacuatiner tubes as needed.

9.18. Once blood collection is complete, discard the entire SteriPath device into a sharps container.

9.19. Scrub the hub vigorously for 15 seconds with an alcohol pad and allow to air dry.

9.20. Attach new needleless adapter cap and flush with 10 ml NS Flush x 2 (total 20 ml) using the push-stop method.

9.21. Properly label each bottle identifying the patient and the collection date/time/site. Apply labels to the bottles vertically.

9.22. Place bottles into a biohazard bag and transport to the laboratory.

10. BLOOD CULTURE COLLECTION – NON-STERIPATH COLLECTION

10.1. In instances where the SteriPath collection device cannot be used (pediatric patients, difficult veins, etc), the blood culture will be collected using standard venipuncture techniques via butterfly or straight needle collection, or by needle and syringe if necessary. Refer to Section 7 above for detailed instructions on collecting a blood culture via venipuncture, but disregard the instructions for SteriPath use.

10.2. Skin preparation and disinfection is the same regardless of the collection method.

10.3. Bottle disinfection, inoculation, and labeling is the same regardless of the collection method.

11. PATIENTS ON ISOLATION:

11.1. Patients on isolation precautions will have a sign on their door alerting staff to the type of isolation and the protocol for entering the room. There will be a cart outside the room with supplies necessary for entering the room. Assemble the required supplies (gown, mask, gloves, phlebotomy supplies etc) prior to entering the room. Do NOT bring in phlebotomy basket or glucose meter.

11.2. Wash hands and forearms thoroughly.

11.3. Don appropriate personal protective equipment (PPE) required for isolation protocol.

11.4. Using proper aseptic technique as described above, perform venipuncture. Label specimens appropriately. Dispose of phlebotomy supplies in a biohazard sharps container, or in red biohazard trash as appropriate.

11.5. Wipe all surfaces (tubes/bottles/etc) with alcohol pads, allow to dry, and place in a biohazard bag for transport.

11.6. Remove PPE and dispose into red biohazard trash. Wash hands and forearms thoroughly.

12. VOLUME REQUIREMENTS AND BOTTLE SELECTION

12.1. Adequate volume is the SINGLE MOST IMPORTANT factor in the laboratory detection of microbes in the blood stream; thus collection methods must be chosen to maximize the volume collected.

12.2. A single blood culture draw is considered one set, and one set typically consists of two bottles (one aerobic and one anaerobic). Depending on the situation (age of the patient, difficulty of draw, etc), it may not be possible to obtain a typical two-bottle set. Regardless of which bottles are used, one should strive to obtain the maximum amount of blood per bottle as possible.

12.3. Specimens should be drawn by direct venipuncture draw into the bottle. If only enough blood can be collected to fill one bottle, inoculate an aerobic bottle only, rather than split the volume between two bottles. It is always better to fill one bottle with the maximum amount of blood, rather than submit two bottles with suboptimal volume in each.

12.4. Refer to chart below to determine bottle type and optimal volume

Type of BacT/Alert Bottle	Color of bottle top	Volume Requirements
Aerobic Bottle	Green *(or blue)*	5 mL minimum, 10 mL optimum & max
Anaerobic Bottle	Orange *(or purple)*	5 mL minimum, 10 mL optimum & max
Pediatric Bottle	Yellow	1 mL minimum, 4 mL optimum & max

13. TIMING OF SPECIMEN COLLECTION

Patient Presentation	What & When to Draw
Acute sepsis, meningitis, osteomyelitis, arthritis, acute untreated bacterial pneumonia, pyelonephritis	Obtain two sets of blood cultures within 30 minutes, collected from different sites, prior to initiating antimicrobial therapy.
Fever of unknown origin	Obtain two to three sets of blood cultures from separate sites initially. After 24-36 hours obtain two more sets prior to temperature spikes if possible.
Suspected Endocarditis or continuous bacteremia of low magnitude.	Acute - Draw three sets of blood cultures from three separate sites during the first one to two hours of evaluation, and begin therapy. Subacute - Draw three sets of blood cultures from three separate sites within 30 minutes on day 1. It may be necessary to draw more sets over several days to recover etiologic agent.
Patients currently on antimicrobial therapy	Draw four to six sets of blood cultures from separate sites over a 48-72 hour period. Collect samples immediately prior to the next dose of antimicrobial agent if possible. *(Note: Blood Culture Bottles with activated charcoal or resin beads may be useful in patients undergoing antimicrobial therapy, as the material in the bottle helps to inactivate the antibiotics).*

14. SPECIAL REQUIREMENTS

14.1. If doctor suspects any of the following organisms, the lab must be contacted PRIOR to drawing of patient's blood to ensure that the proper specimen is obtained. These organisms may require special handling and/or may have to be sent to a reference lab.

14.2. **AFB** - Specimen must be collected by a laboratory phlebotomist trained in blood culture collection techniques. The specimen requirement is a 10mL Isolator Tube (supplies kept in the Microbiology Laboratory).

14.3. **Fungi** - The specimen requirement is a 10mL Isolator Tube (supplies kept in the Microbiology Laboratory).

14.4. **Leptospirosis** - Culture would have to be sent through State Health Department to the CDC. Special papers need to be filled out and State Health Department must give us a special case number. Antibody test can be done through Reference Lab.

14.5. **Bartonella henselae** - Must call State Health to determine if they or CDC performs this test.

15. REFERENCES

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15.2. bioMérieux, Inc. 2002. BacT/ALERT SA, SN, PF, FA, and FN Culture Bottles, Package Inserts.

15.3. Dunne, W. Michael, Jr., Fredrick S. Nolte, Michael Wilson. April 1997. Cumitech 1-B-Blood Cultures III. American Society for Microbiology. p. 10.

15.4. bioMérieux, Inc.. 2002. BacT/ALERT 3D Microbial Detection System, Operator Manual.

15.5. bioMérieux, Inc. 2002. BacT/ALERT SA, SN, PF, FA, and FN Culture Bottles, Package Inserts.

15.6. Isenberg, Henry D. Essential Procedures for Clinical Microbiology. 1998. American Society for Microbiology.

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15.8. Cumitech 1C – Blood Cultures IV. American Society for Microbiology. 2005.